BD Accuri™ C6 Flow Cytometer Instrument Manual

Science is hard. Flow cytometry should be easy.™

TABLE OF CONTENTS

1 INTRODUCTION 1
- 1.1 About This Manual 1
- 1.2 Delivery and Inspection 1
- 1.3 BD Accuri C6 Flow Cytometer Specifications 2

2 SAFETY PRECAUTIONS 3
- 2.1 Safety and Limitations Manual 3
- 2.2 Operational Considerations 3
  - 2.2.1 Ambient Temperature and Humidity 3
  - 2.2.2 Area of Use 3
  - 2.2.3 Approved Reagents 3
  - 2.2.4 Material Safety Data Sheets (MSDS) 4

3 SETTING UP THE BD ACCURI C6 FLOW CYTOMETER 5
- 3.1 BD Accuri C6 Flow Cytometer Layout 5
- 3.2 Configuring the Computer Settings 5
  - 3.2.1 Setting up the Computer 5
  - 3.2.2 Changing the Workstation Computer Settings for Windows® 7 6
  - 3.2.3 Viewing .c6 and .c6t File Extensions in Windows 7 6
- 3.3 Setting Up the BD Accuri C6 Flow Cytometer 7
  - 3.3.1 Setting Up the Reagent Bottles and Fluidics 8
  - 3.3.2 Connecting the Cables 9
- 3.4 Installing Software 10
  - 3.4.1 Installing BD Accuri C6 Software 10
- 3.5 Starting Up the BD Accuri C6 Flow Cytometer 10
- 3.6 Turning Off the BD Accuri C6 Flow Cytometer 11

4 MAINTENANCE 13
- 4.1 Automated Preventive Maintenance Notification 13
- 4.2 Cleaning the SIP 14
- 4.3 Cleaning the Flow Cell 14
  - 4.3.1 Running the Unclog Cycle 14
  - 4.3.2 Running an Extended Clean of the Flow Cell 15
- 4.4 Decontamination Fluid Cycle 15
- 4.5 Cleaning Fluid Cycle 15
- 4.6 Purging Air from the BD Accuri C6 Flow Cytometer 16
- 4.7 Emptying the Waste Container 16
- 4.8 Filling the Fluid Bottles 17
- 4.9 Replacing the Fluidic Bottle Filters 17
- 4.10 Inspecting the Fluidics Lines 18
- 4.11 Replacing the In-line Sheath Filter 18
- 4.12 Replacing the Peristaltic Pump Tubing 19

5 TROUBLESHOOTING AND TECHNICAL SUPPORT 21
- 5.1 Troubleshooting 21
- 5.2 Cleaning and Decontamination—Standard Procedure 24
- 5.3 Limited Warranty 25
- 5.4 Extended Warranties 25
- 5.5 Technical Support 25
- 5.6 Ordering Information 27

FIGURES AND TABLES

Figure 1-1. The BD Accuri C6 Flow Cytometer System 1
Table 1-3. BD Accuri C6 Flow Cytometer Specifications 2
Table 2-1. Approved Reagents 3
Figure 3-1. BD Accuri C6 Flow Cytometer Optics and Fluidics (Top View, Lid Open) 5
Figure 3-2. In-line Sheath Filter 7
Figure 3-3. Interference Filter 7
Figure 3-4. Fluidics harness 8
Figure 3-5. Proper Attachment of Fluidics Lines 9
Figure 3-6. Cable Connections 9
Figure 3-7 SIP and Sample Stage 10
Figure 3-8. Improper Shut down Message 11
Table 4-1. BD Accuri C6 Flow Cytometer Replacement Parts/Reagents List 13
Figure 4-1. Automated Preventive Maintenance Notification Window 14
Figure 4-2. Waste Line Quick Disconnect 17
Figure 4-3. In-line Sheath Filter 18
Figure 4-4. Peristaltic Pumps and Luer Locks 19
Figure 4-5. Removing Peristaltic Pump Retainer Clip 19
Figure 4-6. Sliding Luer Lock Fittings Off of Pump Head 20
Figure 5-1. Browse For Folder Dialog Box 22
Figure 5-2. Update Driver Software – Cytometer, Second Dialog Box 22
Figure 5-3. Windows Security Window 23
Figure 5-4. Update Driver Software – Cytometer, Successfully updated your driver software 23
Figure 5-5. The C6 Software Status Message Box – C6 is connected and ready 23

1 INTRODUCTION

The BD Accuri™ C6 flow cytometer offers an entirely new vision for the role of flow cytometry in life science research. Designed from the ground up to be compact and easy to use, the BD Accuri C6 flow cytometer fits in any lab and is readily accessible to all researchers—experts and novices alike.

Figure 1-1. The BD Accuri C6 Flow Cytometer System

The BD Accuri C6 flow cytometer system includes BD Accuri C6 Software for controlling the instrument, generating statistics, and analyzing results.

1.1 About This Manual

This manual provides information on cytometer requirements and specifications, setup, and maintenance. It also includes common troubleshooting help and service information.

- For information on using BD Accuri C6 Software, see the *BD Accuri C6 Software User Guide.*
- For information on installing and using the BD CSampler™, see the *BD Accuri CSampler Software User Guide.*

1.2 Delivery and Inspection

Carefully inspect all cartons upon receipt of the cytometer. If there are any signs of mishandling or damage, file a claim with the carrier immediately. If the shipment is separately insured, file a claim with the insurer.

ITEMS **NOT** PROVIDED

- Surge suppressor or uninterruptible power supply (UPS). A model rated at a minimum of 1000 VA is recommended for use with the cytometer, computer, and monitor.
- Microsoft® Office™ or equivalent
- Anti-virus software. This system is intended for use as a stand-alone station, not as part of a network.

1.3 BD Accuri C6 Flow Cytometer Specifications

Table 1-3. BD Accuri C6 Flow Cytometer Specifications

Laser Excitation	488 nm (rated at 20,000 hr life) 640 nm (rated at 20,000 hr life)
Laser Profile	10 x 75 µm
Laser Power	14.7 mW 640 nm Diode Red Laser 20 mW 488 nm Solid State Blue Laser
Scatter Detection	Forward (0 degrees, +/-13) Side (90 degrees, +/-13)
Emission Detection	4 colors, standard optical filters • FL1 533/30 nm (e.g. FITC/GFP) • FL2 585/40 nm (e.g. PE/PI) • FL3 > 670 nm (e.g. PerCP, PerCP-Cy™5.5, PE-Cy7) • FL4 675/25 nm (e.g. APC)
Optical Alignment	Fixed alignment
Flow Cell	200 µm ID quartz capillary
Minimum Detectable Particle Size	0.5 µm
Minimum Sample Volume	300 µL (in 12 x 75 mm tube)
Pre-Set Flow Rates and Core Sizes	Slow: 14 µL/min, 10 µm core Medium: 35 µL/min, 16 µm core Fast: 66 µL/min, 22 µm core
Custom Sample Flow Rates	10 – 100 µL/min
Custom Core Diameter	5 – 40 µm
Recommended Sheath Fluid	0.22 µm filtered, deionized (DI) water
Maximum Events Per Sample	1 million events per well
Florescence Sensitivity, MESF*	FITC < 150; PE < 100
Scatter Resolution	Resolves human peripheral blood lymphocytes, monocytes, and granulocytes
Fluorescence Linearity	2 ± 0.05% for chick erythrocyte nuclei (CEN)
Fluorescence Precision	≤ 3% CV for CEN
Data Acquisition Rate	10,000 events/second, maximum
Power Requirements	100 - 240 VAC, 50/60 Hz
Typical Power Consumption	154 VA
Heat Output	240 Btu/hour maximum output
Operating Ranges	15 - 30° C; < 80% relative humidity
Instrument Size	11”H x 14.75”W x 16.5”D (27.9 x 37.5 x 41.9 cm)
Foot Print with Fluid Bottles	11”H x 21.5”W x 16.5”D (27.9 x 54.6 x 41.9 cm)
Weight	30 lbs (13.6 kg)
Fluid bottle capacity	2 L sheath fluid, 2 L waste, 250 mL cleaner, 250 mL decontamination
Signal Processing	24-bit datapath
Computer Interface	USB 2.0
Warranty	1 year

*MESF values determined using Thermo Scientific Cyto-Cal™ Multifluor Plus Violet Beads.

2 SAFETY PRECAUTIONS

2.1 Safety and Limitations Manual

Refer to the BD Accuri C6 Safety and Limitations Manual (PN 7820091) for complete safety information.

2.2 Operational Considerations

When setting up the cytometer, note the details in the following sections to maximize the instrument's performance.

2.2.1 Ambient Temperature and Humidity

Operating temperature of the cytometer is between 15°C and 30°C (59°F and 86°F). Operating humidity is < 80% relative humidity.

2.2.2 Area of Use

The cytometer is designed to fit on a laboratory bench top. Keep a perimeter of at least six (6) inches (15 cm) around the instrument and computer for proper ventilation and to protect the instrument and computer from liquid spills.

Take special care while handling fluids around the cytometer. Ensure that uncontained fluids do not enter the instrument or computer. Clean up spills immediately. Never place anything on top of the cytometer, including sample tubes and racks.

Turn off the instrument and unplug the power cord before manually cleaning the cytometer.

2.2.3 Approved Reagents

Only approved fluids should be used in the operation or cleaning of this instrument. See the following table for a list of approved reagents.

Table 2-1. Approved Reagents

Fluid	*Description*
Sheath fluid	0.22 µm filtered, deionized (DI) water with or without bacteriostatic concentrate solution (PN 653156)
Cleaning solution	Cleaning concentrate solution (PN 653157) diluted according to package instructions
Decontamination solution	Decontamination concentrate solution (PN 653154) diluted according to package instructions
Extended flow cell clean	Extended flow cell cleaning solution (PN 653159)

Do not use household bleach as a decontamination solution. It contains fluorescent whitening agents that may interfere with fluorescence detection.

2.2.4 Material Safety Data Sheets (MSDS)

To obtain an MSDS, visit www.AccuriCytometers.com or contact:

- In the USA:
  Email TechSupport@AccuriCytometers.com
  Call Accuri Technical Support at 1.734.994.8000
- Outside the USA
  Visit www.AccuriCytometers.com for country-specific contact information.

3 SETTING UP THE BD ACCURI C6 FLOW CYTOMETER

Perform all of the procedures in this chapter before running any experimental samples.

3.1 BD Accuri C6 Flow Cytometer Layout

The cytometer is composed of fluidics, optics, and electronics subsystems that work together to analyze cell populations. The following figures show the major components of the fluidics and optics subsystems.

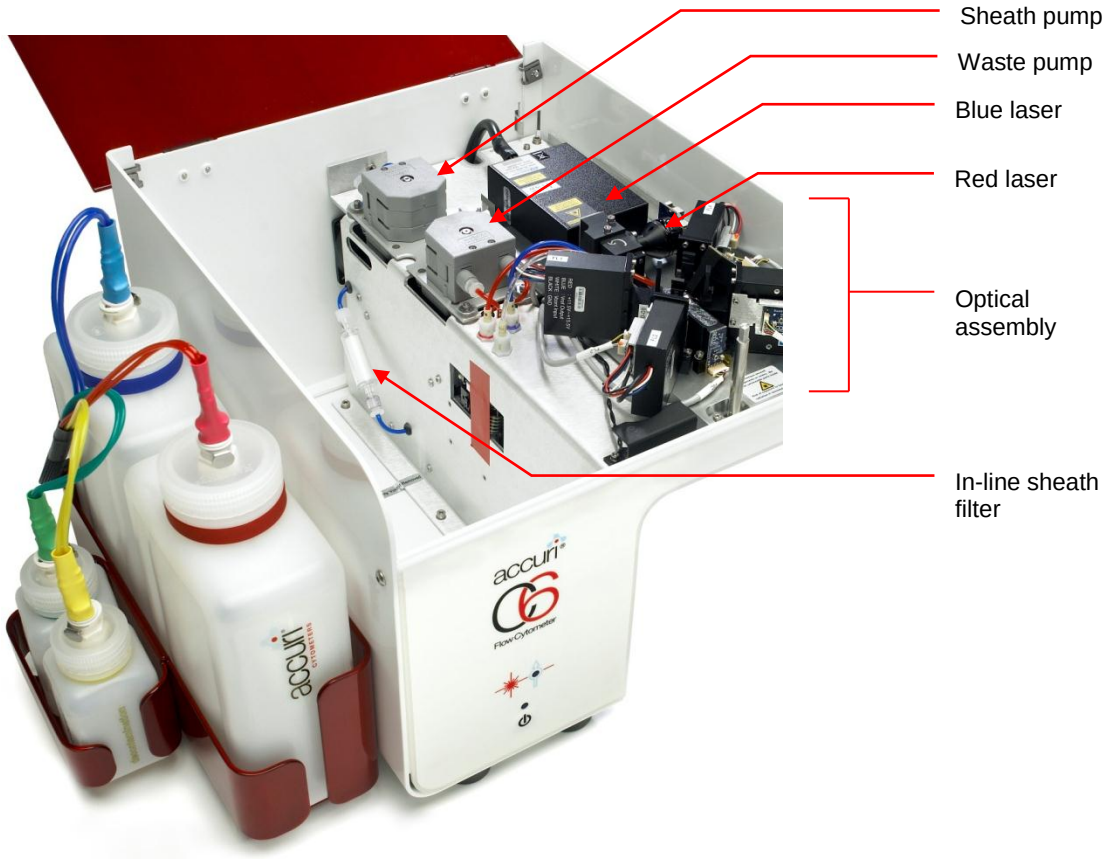

Figure 3-1. BD Accuri C6 Flow Cytometer Optics and Fluidics (Top View, Lid Open)

3.2 Configuring the Computer Settings

3.2.1 Setting up the Computer

Set up the computer, monitor, keyboard, and mouse according to manufacturer's instructions. Computers come preinstalled with Windows® 7 Professional. Contact Accuri Technical Support for assistance with other operating systems.

Install the software on the computer before connecting the USB cable to the cytometer.

NOTE: We recommend making the following changes to the C6 workstation computer in order to achieve the best possible work environment and to avoid unexpected software computer issues that could potentially result in a loss of data.

3.2.2 Changing the Workstation Computer Settings for Windows® 7

Skip this section if your computer has BD Accuri C6 Software preinstalled.

1. Go to Start (circular icon located in the lower left-hand corner) / select Control Panel.
2. Select “Appearance and Personalization”
3. Turn off the Sleep Settings.
    a. From the column on the left, select “System and Security” then “Power Options”
    b. Select “Change when the computer sleeps” from the left hand margin (alternatively select “change plan settings” from the main window).
    c. From the Edit Plan Settings window, change the “Put the computer to sleep” setting to “Never” using the drop-down function (we also suggest setting the “Turn off the display” setting to Never).
    d. Select “Save Changes”. Then close the control panel window.
4. Optimize Monitor Display Settings.
(From Start / Control Panel / Appearance and Personalization)
    a. Select “Display”, select “Adjust Resolution” from the column on the left. (Alternatively select “Adjust screen resolution” directly from the Display section of the main Appearance and Personalization window). Then select (highlight) Monitor 1.
    b. Ensure the Resolution is set to the maximum setting or to at least 1152 x 864. Change the setting using the slider under the “Resolution” field’s drop down menu, if necessary.
    c. Select “Apply”, then select “OK” and close the Personalization window.

3.2.3 Viewing .c6 and .c6t File Extensions in Windows 7

To view hidden file extensions which distinguish BD Accuri C6 Software files (.c6) from BD Accuri C6 Software template files (.c6t):

1. Click on the Windows *Start* button and select *Control Panel* from the pop-up menu.
2. Select “Tools” from the menu bar.
3. Click on “View” tab
4. Click “Show hidden files and folders”
5. Deselect “Hide extensions for known file types”

6. Select “Apply,” then “OK” to save changes and close dialog box.

3.3 Setting Up the BD Accuri C6 Flow Cytometer

1. Remove the cytometer and other components from the shipping boxes.
2. With the front of the cytometer facing toward you, lift the top cover.
3. Verify that the in-line sheath filter is installed. If it is not installed, see section 4.11 for instructions on replacing the filter.

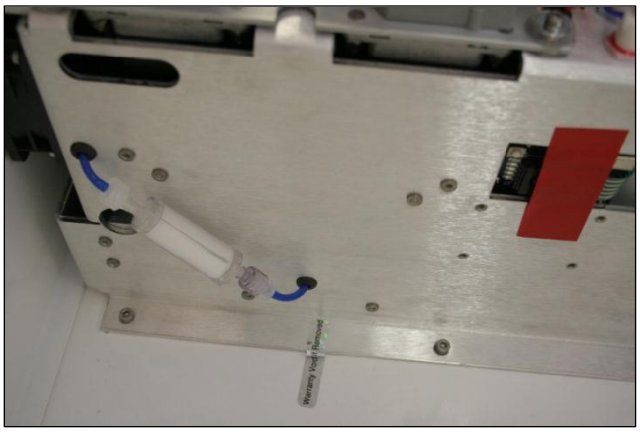

Figure 3-2. In-line Sheath Filter

4. Verify that there are no disconnected electronic cables on the optics assembly.
5. Verify that the interference filters are firmly seated (Figure 3.3). The standard interference filters are labeled as follows:
   - FL1—533/30
   - FL2—585/40
   - FL3—670LP
   - FL4—675/25

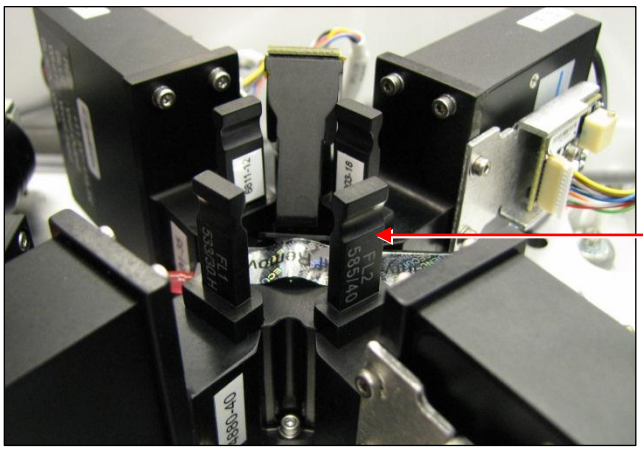

Figure 3-3. Interference Filter

Never run the cytometer without interference filters installed. Running the cytometer without the filters may damage the instrument.

6. Gently close the cytometer cover. Make sure the cover is securely positioned. If the cover is not closed securely, the cytometer will not function.
7. Place the fluidics bottle tray in a convenient location near the perimeter of the cytometer.

Do not place the fluidics bottle tray on top of the cytometer.

3.3.1 Setting Up the Reagent Bottles and Fluidics

1. Fill the sheath bottle with sheath fluid.
2. Dilute the decontamination concentrate (PN 653154) and cleaning concentrate (PN 653157) solutions as instructed and fill decontamination and cleaner bottles, respectively.

Close the bottles securely. Do not over tighten.

3. Add approximately 100 mL of 0.5% NaOCl to the Waste bottle.
4. Place the bottles in the fluidics bottle tray.

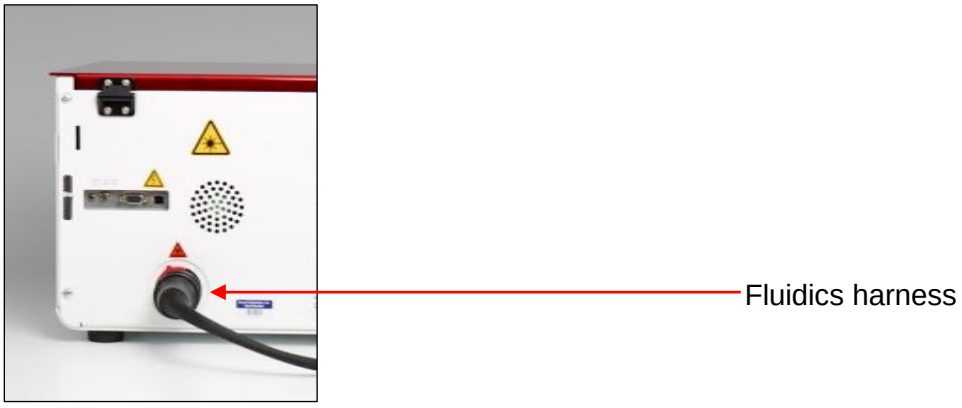

Figure 3-4. Fluidics harness

5. Slide the red tab on the black end of the fluidics harness to the unlock position and attach the black end of the fluidics harness to the back of the cytometer.
6. Slide the red tab to the lock position.
7. Attach the other end of the fluidics harness to the appropriate bottles:
   - Red line—waste bottle
   - Blue line—sheath bottle
   - Yellow line—decontamination bottle
   - Green line—cleaner bottle

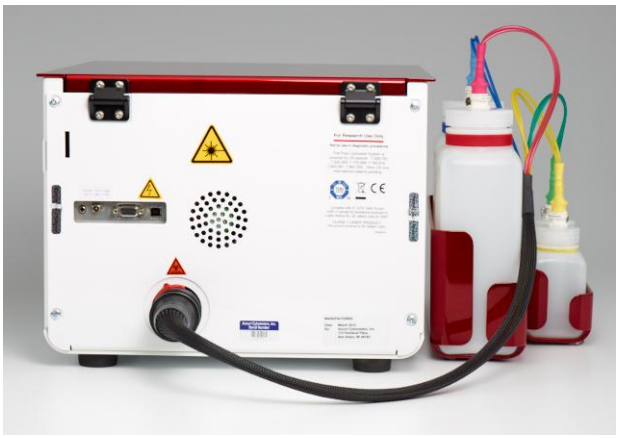

Figure 3-5. Proper Attachment of Fluidics Lines

3.3.2 Connecting the Cables

1. Plug the cord from the power supply into the back of the cytometer.

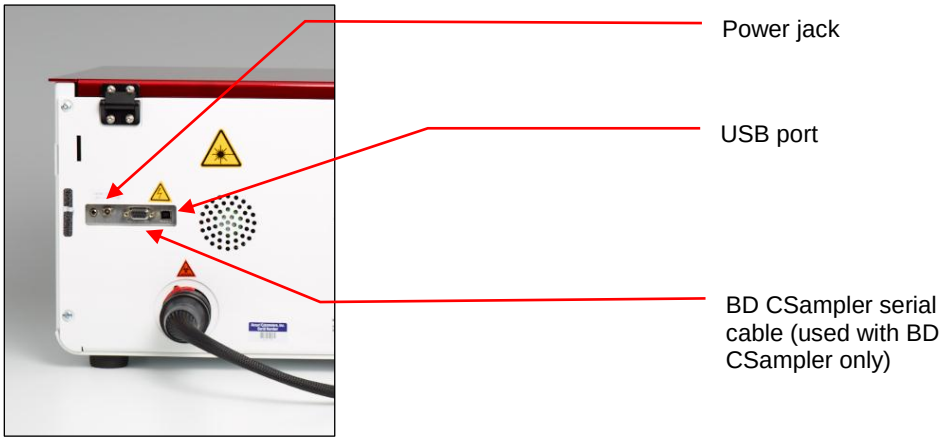

Figure 3-6. Cable Connections

2. Attach the black power cord to the power supply and plug into a standard outlet.

   Only operate this instrument from a supply source that incorporates a third-wire protective grounding conductor. A surge suppressor or power strip is recommended.

3. Connect the USB cable to the back of the cytometer.

   Do not plug the USB cable into the computer yet.

   To prevent putting pressure on the fluidics lines and USB cable connection, do not place the cytometer against a wall or another object.

3.4 Installing Software

This section provides details on installing BD Accuri C6 Software and the BD Accuri C6 flow cytometer driver. In most cases, BD Accuri C6 Software will be pre-installed. If software is installed, skip to section 3.5.

3.4.1 Installing BD Accuri C6 Software

1. Ensure the USB cable is *not* connected between the computer and cytometer.
2. Start up the computer and monitor.
3. Insert the provided BD Accuri C6 Software CD. In the CD directory, double-click on BD Accuri C6 Software Installer.exe.
4. Follow the instructions in the installation wizard.

3.5 Starting Up the BD Accuri C6 Flow Cytometer

1. Check fluid levels in all bottles, ensuring that the Waste is empty and the Sheath, Cleaner and Decontamination bottles are full.
2. Gently push the sample stage back, remove and discard the SIP cover and place a tube containing at least 2 mL of 0.22 µm filtered, DI water on the SIP.

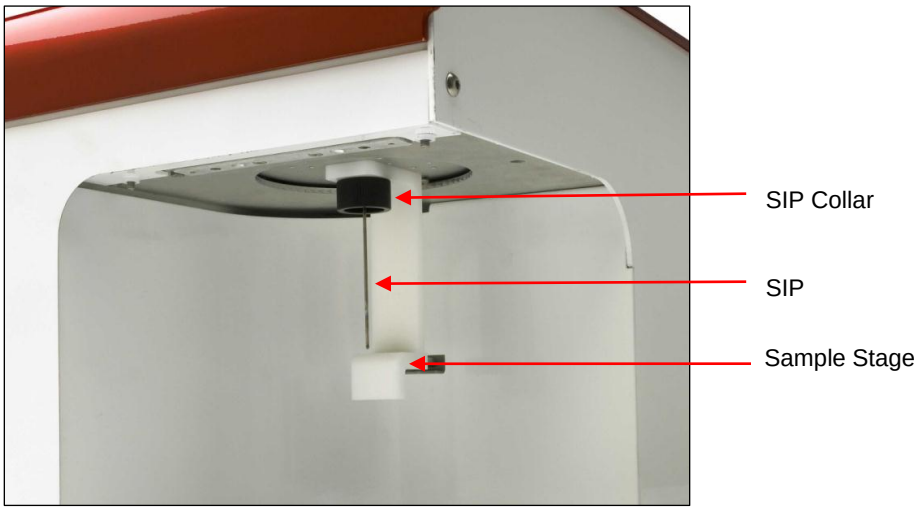

Figure 3-7 SIP and Sample Stage

NOTE: The sample stage can accommodate several types of tubes, including any brand of 12x75 mm tube and most microcentrifuge tubes

3. While holding the sample tube in place, pull the sample stage forward to support the tube. The stage clicks into place.

The sample tube does not form a seal at the top of the SIP. While inserting the SIP in the tube, be careful not to bend or catch the SIP on the outside of the tube.

4. Firmly press the power button on the front of the unit to turn on the cytometer. During start up, the BD Accuri C6 Software Traffic Light turns yellow and the cytometer pumps start to run. When powered on, the cytometer automatically flushes the fluidics lines with sheath. This process takes approximately five minutes.

   Do not open the lid of the cytometer during the startup process. Opening the lid interrupts the laser warm-up and extends the time before data can be acquired.

5. When the BD Accuri C6 Software Traffic Light turns green and BD Accuri C6 Software displays the message *C6 is connected and ready,* run 0.22 µm filtered, DI water for at least 15 minutes before processing samples.

6. Validate cytometer performance. See the *BD Accuri C6 Software User's Guide* for details on instrument validation using the validation beads provided with the cytometer.

3.6 Turning Off the BD Accuri C6 Flow Cytometer

1. Place a tube with 2 mL of decontamination solution on the SIP.
2. Select an empty data well in the Collect Tab of BD Accuri C6 Software.
3. Set the time limit for two minutes and the fluidics speed to fast.
4. Click on the *RUN* button.
5. Once the run is finished, remove the tube of decontamination solution from the SIP.
6. Place a tube with 2 mL of 0.22 µm filtered, DI water on the SIP and advance to any empty data well.
7. Set the time limit for two minutes and fluidics to *Fast*.
8. Click on the *RUN* button.
9. When the run is finished, leave the tube on the SIP.
10. Press the power button. The shutdown cycle runs for approximately 15 minutes, then the cytometer automatically powers off.

    Depressing the power button for five seconds or longer bypasses the auto-fluidic shutdown cycle. If the cytometer is shut down in this manner, BD Accuri C6 Software displays the following message during the next start up:

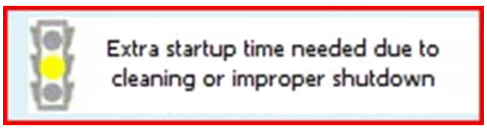

Figure 3-8. Improper Shut down Message

When this occurs, the cytometer takes additional time to recover and return to the green light ready state.

NOTE: It is not necessary to exit BD Accuri C6 Software or turn off the computer before turning off the cytometer.

4 MAINTENANCE

Changing the in-line sheath filter, fluidic bottle filters, and peristaltic pump tubing every two months is recommended. Heavy use of the cytometer may warrant more frequent changes.

The following table lists the parts and reagents that are required for regular maintenance.

Table 4-1. BD Accuri C6 Flow Cytometer Replacement Parts/Reagents List

Part Number	*Description*
653146	Peristaltic Pump Tubing (Set of 2)
653147	Sheath bottle filter (1 large) and Decon/Cleaner Bottle Filters (2 small)
653148	In-line Sheath Filters (1 ea.)
653149	BD Accuri C6 Flow Cytometer 1-year Maintenance Kit contains: (12) Peristaltic Pump Tubing, (6) Sheath Bottle Filter, (12) Decontamination/Cleaner Bottle Filters, and (6) In-line Sheath Filters
653150	BD Accuri C6 Flow Cytometer 2-Month Maintenance Kit contains (2) Peristaltic Pump Tubing, (1) Sheath Bottle Filter, (2) Decon/Cleaner Bottle Filters and (1) In-line Sheath Filter
653154	Decontamination Concentrate Solution (makes 200 mL)
653155	Decontamination Concentrate Solution (makes 1 L)
653156	Bacteriostatic Concentrate Solution (for treating 10 L Sheath Fluid) (10 vials)
653157	Cleaning Concentrate Solution (makes 1 L)
653158	BD Accuri C6 Flow Cytometer Fluid Kit: contains concentrate for making 1 L each of Cleaning and Decontamination Solutions, Bacteriostatic Concentrate for treating 10 L of Sheath, and 8 mL of Extended Flow Cell Clean
653159	Extended Flow Cell Clean Solution
653160	BD Accuri C6 Flow Cytometer Starter Kit: Contains concentrate to make 1 L each of Cleaning and Decontamination Solutions, Bacteriostatic Concentrate to treat 10 L of Sheath,8 mL of Extended Flow Cell Clean, and 4 mL of both 6- and 8-Peak Validation Beads

Visit www.accuricytometers.com for a complete list of available parts and reagents.

4.1 Automated Preventive Maintenance Notification

An automated notification and reminder system is contained in the BD Accuri C6 Software to alert the user to perform the recommended preventive maintenance as described in this manual. This includes removal and replacement of the peristaltic pump tubing, in-line sheath filter and the fluidic bottle filters.

BD Accuri C6 Software will trigger the notification based on the volume of sheath run through the system or on the date since maintenance was last performed. BD Accuri C6 Software will display this notification based on whichever trigger is reached first. The notification appears as a pop-up window that allows the user to choose between 'Remind me later' or 'Maintenance is complete'.

Selecting 'Maintenance is complete' will re-start both triggers.

Selecting 'Remind me later' will set the notification to remind the user in 5 days.

Maintenance Required
Avoid high background counts and blocked valves!
Inform the maintenance supervisor to replace the following:
1. Reagent bottle filters
2. In-line sheath filter
3. Tubing in the peristaltic pumps
Remind me later
Maintenance is complete
OK

Figure 4-1. Automated Preventive Maintenance Notification Window

4.2 Cleaning the SIP

Run the backflush cycle as needed to clean the SIP and remove clogs at the base of the SIP.

1. Place a blotter or empty sample tube under the SIP to catch dripping fluid.
2. Do one of the following:
   - Click on the *Backflush* button in the *Collect* tab.
   - Select *Instrument > Run Backflush Cycle*

4.3 Cleaning the Flow Cell

Clean the flow cell as a part of regular maintenance or to correct performance issues of the cytometer.

4.3.1 Running the Unclog Cycle

The unclog cycle purges the flow cell of debris.

1. Remove the sample tube from the SIP and place a blotter or empty sample tube under the SIP to catch dripping fluid.
2. Do one of the following:

- Click on the *Unclog* button in the *Collect* tab.
- Select *Instrument* > *Run Unclog Cycle.*

4.3.2 Running an Extended Clean of the Flow Cell

For a thorough cleaning of the flow cell, perform an extended flow cell clean. During extended flow cell cleaning, the flow cell fills completely with cleaning solution from the sample tube on the SIP. This cycle automatically shuts down the cytometer with cleaning reagent in the flow cell, allowing the flow cell to soak.

1. Place a tube with at least 500 µL of Extended Flow Cell Clean Solution (PN 653159) on the SIP.

   Never run the Extended Clean of Flow Cell cycle without a tube containing at least 500 µL of fluid.

2. Select *Instrument* > *Extended clean of flow cell*.
3. After the cytometer is automatically shut down, allow the cytometer to rest for at least 30 minutes (up to overnight for a more thorough cleaning).
4. Replace the tube of Extended Flow Cell Clean Solution with a tube of 2 mL 0.22 µm filtered, DI water.
5. Restart the cytometer. The cytometer performs a longer fluidics startup cycle and the Software displays the message *Extra startup time needed due to cleaning or improper shutdown*. This longer cycle purges cleaning reagent from the flow cell.
6. Run 0.22 µm filtered, DI water for 10 minutes on fast speed.
7. Operate the cytometer as usual.

4.4 Decontamination Fluid Cycle

The BD Accuri C6 automatically runs the decontamination fluid cycle when the system is shut down normally. The decontamination fluid cycle lasts about 15 minutes.

To manually run the decontamination fluid cycle:

1. Place a tube with 2mL of 0.22 µm filtered, DI water on the SIP.
2. Select *Instrument* > *Run decontamination fluid cycle*.

4.5 Cleaning Fluid Cycle

The cleaning fluid cycle pulls cleaner fluid from the cleaner tank and runs it through the fluidic lines of the fluidics system. After filling the system with cleaner fluid, the cleaning fluid cycle purges the cytometer with fresh sheath fluid and performs a backflush. This cycle takes about five minutes.

1. Place a tube of cleaning solution (PN 653157) on the SIP.
2. Select *Instrument* > *Run cleaning fluid cycle*.

4.6 Purging Air from the BD Accuri C6 Flow Cytometer

Purge air from the cytometer if any of the following is true:

- The cytometer is being set up for the first time.
- The cytometer has not been used for more than two days.
- A sample has been run dry or the cytometer has been running without sheath in the sheath bottle.

To purge air from the cytometer:

1. Place a tube containing sheath on the SIP.

   NOTE: If performing an air purge at any time other than initial setup, skip steps 2-3.

2. Select *Instrument* > *Run decontamination fluid cycle*.
3. Wait approximately 15 minutes for the decontamination fluid cycle to complete.
4. Set the run time in the software control panel to five minutes and click on the *Run* button. Allow the cytometer to run until it stops automatically.

   NOTE: The peristaltic pumps will make a rhythmic sound while running. This is normal.

5. Click on the *Run* button again, wait for 30 seconds, and click on the *Pause* button to stop the pumps.
6. Repeat step 5 two more times to purge bubbles from the system.

4.7 Emptying the Waste Container

Empty the waste bottle daily or when prompted by BD Accuri C6 Software to prevent spillover.

Biological samples are potentially dangerous and/or life threatening. Adhere to proper handling procedures for samples and reagents at all times. Wear appropriate laboratory attire such as protective gloves and eyewear during this procedure.

1. Disconnect the quick connect waste line from the top of the waste bottle.

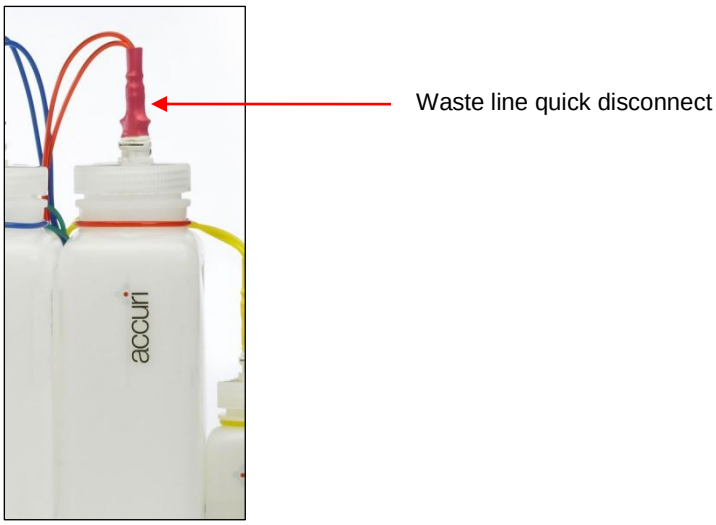

Figure 4-2. Waste Line Quick Disconnect

2. Remove the bottle lid.
3. Dispose of waste according to local regulations.
4. Add approximately 100 mL of 0.5% NaOCl to the Waste bottle.
5. Replace the lid.
6. Reattach the waste line to the top of the bottle.

4.8 Filling the Fluid Bottles

Before turning on the cytometer, visually check all the bottles and fill the sheath, decontamination, and cleaning solution bottles as needed.

NOTE: The cytometer is a non-pressurized system. If necessary, any of the bottles can be opened while the cytometer is powered on.

NOTE: BD Accuri C6 Software displays a message when the fluid bottles need attention or if a bottle level sensor is improperly connected.

4.9 Replacing the Fluidic Bottle Filters

Each of the sheath, cleaner, and decontamination bottles contain a disk filter. Replace these filters (PN 653147) every two months. Wear appropriate safety attire such as protective gloves and eyewear when performing this procedure.

1. Disconnect the quick connect lines from the top of each bottle.
2. Carefully remove the lid from each bottle.
3. Disconnect the filter luer lock at the end of the fluidic tubing. Discard the filter according to standard laboratory protocols and regulations.
4. Replace the filter with the appropriate part:
   - Sheath bottle—large disk filter
   - Cleaner and decontamination bottles—small disk filter
5. Reassemble the bottles and reconnect the quick connect lines.

6. Place a tube with 2 mL of 0.22 µm filtered, DI water on the SIP and run for one minute.

4.10 Inspecting the Fluidics Lines

Check the cytometer periodically to ensure there are no fluid leaks or crimped lines.

1. Turn off the cytometer.
2. Open the top cover.
3. Visually inspect for fluid leaks by looking for small pools of liquid near any of the visible luer lock connections.
4. Visually inspect for dried residue or slight discoloration of the metal fluidics tray plate, making sure to look under the glovebox and under the cytometer.
5. If any evidence of a leak is seen, contact Accuri Technical Support immediately. Do not attempt to repair the instrument.

Any evidence of fluid coming from, or near, a red or clear line should be considered a biological hazard. Do not attempt to clean up the fluid without gloves and other safety attire. Dispose of all cleaning materials as if biologically contaminated.

4.11 Replacing the In-line Sheath Filter

Replace the in-line sheath filter (PN 653148) every two months. If the cytometer is used daily for more than a few hours, replace the filter monthly. Wear appropriate safety attire such as protective gloves and eyewear when performing this procedure.

1. Turn off and unplug the cytometer.
2. Gently lift the cytometer cover.
3. Remove the plastic storage compartment.
4. Twist the luer locks on the in-line sheath filter to disconnect the locks.
5. Discard the filter according to standard laboratory protocols and regulations.

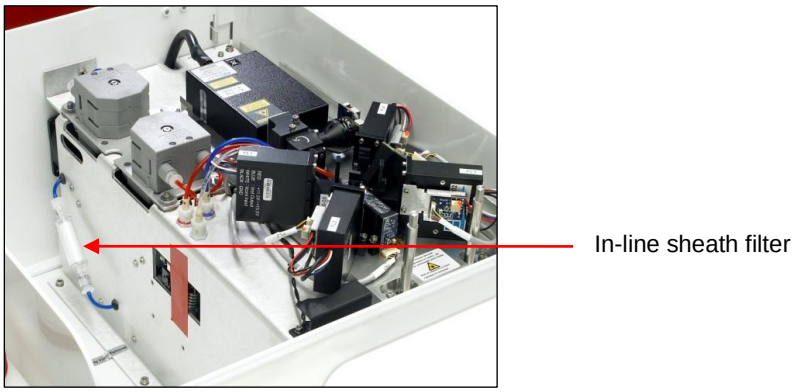

Figure 4-3. In-line Sheath Filter

6. Install a new in-line sheath filter (PN 653148). This filter has a male and female end to ensure that it can only be installed in the correct orientation.
7. Replace the storage compartment.

8. Gently close the cytometer cover.
9. Place a sample tube with 2mL of 0.22 µm filtered, DI water on the SIP.
10. Plug in and turn on the cytometer.
11. Purge any air from the instrument (see section 4.6).

4.12 Replacing the Peristaltic Pump Tubing

Replace the pump tubing (PN 653146) every two months. Replace both pieces of tubing at the same time. Tubing comes in contact with biological samples and therefore should be considered hazardous. Wear gloves and appropriate safety gear during this procedure.

There are two peristaltic pumps: a sheath pump and a waste pump.

1. Turn off and unplug the cytometer.
2. Lift the cytometer lid.
3. Disconnect the luer locks from the peristaltic pumps by unscrewing them. Blue tubing is connected to the sheath pump and red tubing to the waste pump.

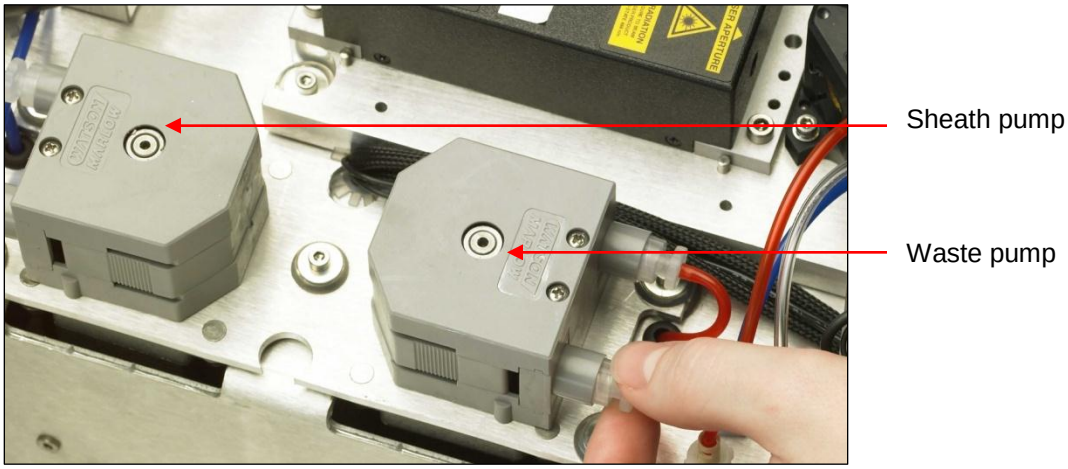

Figure 4-4. Peristaltic Pumps and Luer Locks

4. Squeeze the grip marks on the pump element retainer clip to remove the clip.

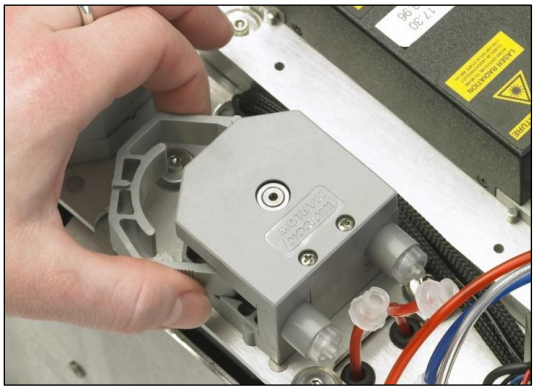

Figure 4-5. Removing Peristaltic Pump Retainer Clip

5. Pull the luer lock connectors outward and slide the luer lock fittings off the pump head.

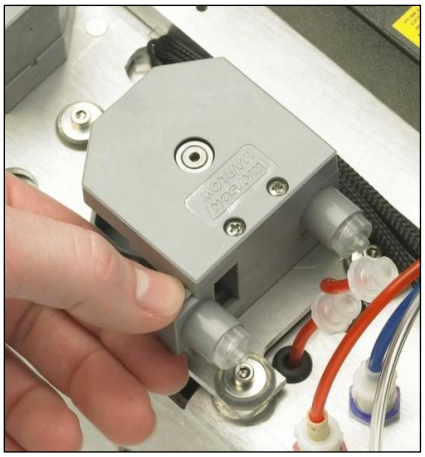

Figure 4-6. Sliding Luer Lock Fittings Off of Pump Head

6. Remove the peristaltic pump tubing and discard according to standard laboratory protocols and regulations.
7. Replace with new peristaltic pump tubing (PN 653146) by sliding the luer lock fittings on the pump head.
8. Snap the pump element retainer clip into place. Reconnect the luer lock fittings on the outside of the pumps. Ensure the blue and red tubes are not crimped or over tightened.

   Ensure that the luer lock fittings are connected to the correct tubing elements.
9. Gently close the cytometer cover.
10. Place a sample tube with sheath on the SIP.
11. Plug in and turn on the cytometer.
12. Purge air from the cytometer (see section 4.6).

5 TROUBLESHOOTING AND TECHNICAL SUPPORT

This section provides troubleshooting and technical support information.

5.1 Troubleshooting

This section contains basic troubleshooting guidelines. Follow the procedures listed below. If problems persist after performing troubleshooting, contact Accuri Technical Support.

The cytometer and/or computer does not power on.

1. Make sure the power supplies and cords are plugged into an appropriate outlet.
2. Check the outlet to make sure it is functioning properly.

The acquisition controls on BD Accuri C6 Software do not show a green light and remain a gray color after the cytometer is powered on.

1. Make sure the USB cable is properly attached to the cytometer and computer. It may take several seconds for the port to recognize the cytometer.
2. Switch the USB cable to a different port on the computer.
3. Make sure the BD Accuri C6 flow cytometer driver was properly installed on the computer by doing the following, in order:

   - Double-click the BD Accuri C6 Software icon on your computer desktop to open BD Accuri C6 Software. (See the *BD Accuri C6 Software User's Guide* or *Quick Start Guide Collection* for instructions on using BD Accuri C6 Software.)
   - Connect the USB cable from the C6 to one of the computer USB ports.

   CAUTION: Do not plug the USB cable for the cytometer into any USB ports on the computer monitor.

   NOTE: Windows will attempt to auto install the BD Accuri C6 driver. The attempt will end with a message stating "Device driver was not successfully installed." This is as expected.

   - After the C6 Device driver fails to auto install, Click the "Start" button (round button with the Windows logo on the lower left of the screen).
   - Select *Devices and Printers.* You should see an icon labeled *Cytometer* that has a yellow symbol with an exclamation point in it.
   - Right-click on the *Cytometer* icon and select *Properties*. Once the properties window opens, select the *Hardware* tab.
   - With the hardware tab open, click the *Properties* button.
   - Click the *Change Settings* button.
   - When the settings window opens, click the *Update Driver* button.
   - When the driver update window opens, select *Browse my computer for driver software*.

- Navigate to the location of the driver software and specify the following path:

C:\Program Files\BD Accuri\BD Accuri C6 Software\libraries

Then click on the *OK* button to enter the selected path into the Browse window.

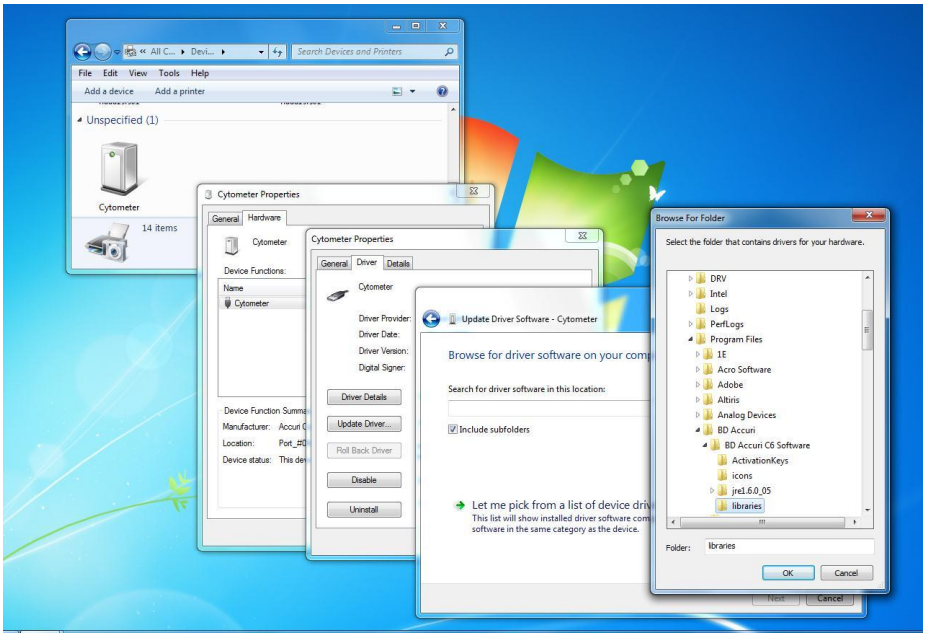

Figure 5-1. Browse For Folder Dialog Box

- Click the *Next* button.

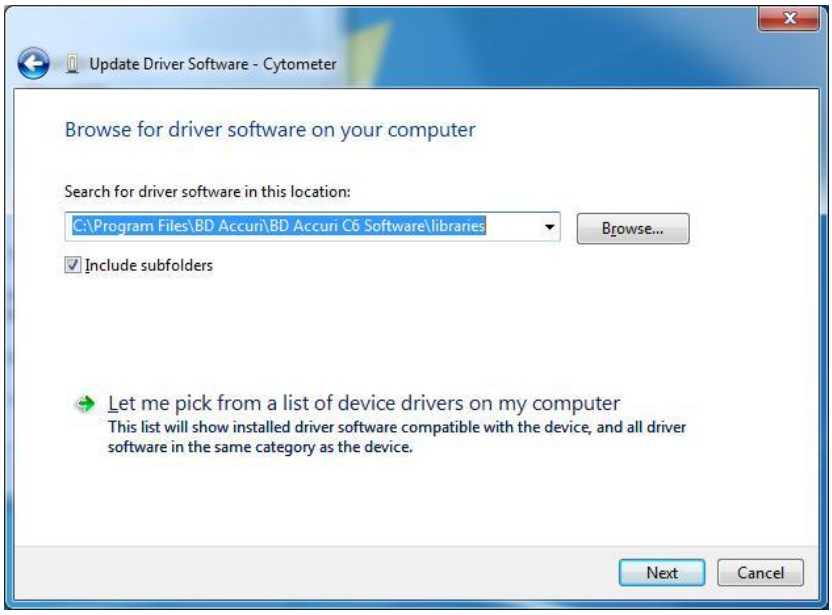
Update Driver Software - Cytometer
Browse for driver software on your computer
Search for driver software in this location:
C:\Program Files\BD Accuri\BD Accuri C6 Software\libraries
Browse...
Include subfolders
Let me pick from a list of device drivers on my computer
This list will show installed driver software compatible with the device, and all driver software in the same category as the device.
Next
Cancel

Figure 5-2. Update Driver Software – Cytometer, Second Dialog Box

- When the Windows Security dialog box shown below opens, click on *Install this driver software anyway*.

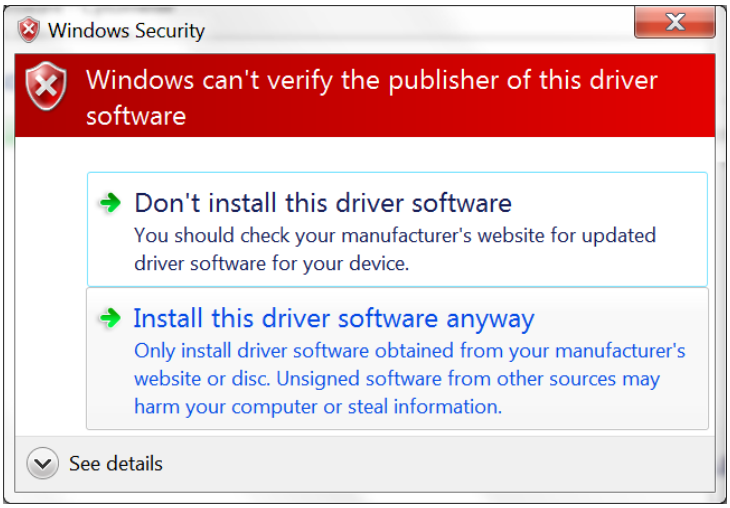
Windows Security
Windows can't verify the publisher of this driver software
Don't install this driver software
You should check your manufacturer's website for updated driver software for your device.
Install this driver software anyway
Only install driver software obtained from your manufacturer's website or disc. Unsigned software from other sources may harm your computer or steal information.
See details

Figure 5-3. Windows Security Window

- Windows will start the installation of the driver for the C6 cytometer. Once completed, you have successfully installed the driver software. When prompted, click the *Close* button.

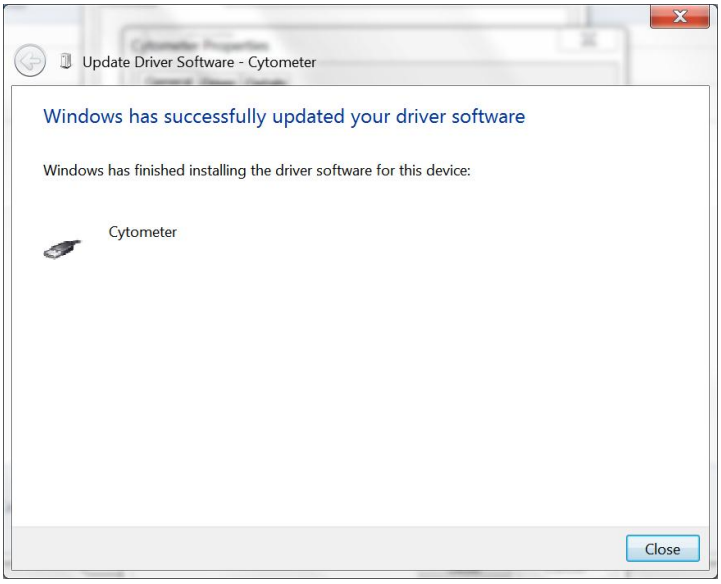
Update Driver Software - Cytometer
Windows has successfully updated your driver software
Windows has finished installing the driver software for this device:
Cytometer
Close

Figure 5-4. Update Driver Software – Cytometer, Successfully updated your driver software

- With the C6 driver installed and the USB cable properly connected, BD Accuri C6 Software will indicate that it recognizes the C6 by displaying a green traffic light and the message “C6 is connected and ready”.

Figure 5-5. The C6 Software Status Message Box – C6 is connected and ready

Data does not look as expected.

1. Pause and restart the sample.
2. Run a backflush or unclog (see sections 4.2 and 4.3.1).
3. Run the validation beads (PN 653144 or 653145) (see the *BD Accuri C6 Software User Guide* for details). If the beads look acceptable, there may be a problem with the sample. If the beads are out of performance specification, perform a decontamination fluid cycle (see section 4.4) and run the beads again. If they are still out of specification, call Accuri Technical Support.

The event rate drops off while running a sample, but the cytometer is not clogged.

1. Place a tube of cleaning solution on the SIP and run for five minutes.
2. Run 0.22 µm filtered, DI water for one minute on Fast fluidics speed.
3. Check to see if the event rate is restored.

The cytometer pumps are running normally, but BD Accuri C6 Software is not acquiring data.

1. Check the levels in the sheath and waste bottles.
2. Check to see if any of the external fluidic lines are crimped.
3. Run a backflush to see if the SIP is clogged (see section 4.2).
4. Make sure the peristaltic pump tubing and retainer clips are properly attached (see section 4.12).
5. Restart BD Accuri C6 Software.

The cytometer pumps are running continually.

1. Check for a large air bubble in the in-line sheath filter. If so, replace the filter (see section 4.11).
2. There could be a clog in the line. Run backflush or unclog.
3. The peristaltic pump tubing may need to be replaced (see section 4.12).

For additional technical support, view the frequently asked questions (FAQs) at www.AccuriCytometers.com/customer_support/faqs/ or contact Accuri Technical Support if a problem persists.

5.2 Cleaning and Decontamination—Standard Procedure

This procedure should be performed when preparing the system for storage or shipping it for service. Contact Accuri Technical Support for more information on shipping. Visit www.AccuriCytometers.com for information on service contracts.

Take appropriate safety measures such as wearing gloves and proper laboratory attire while performing this procedure.

1. Turn on the Cytometer and open the BD Accuri C6 Software.

2. Place a sample tube containing 3 mls of diluted Decontamination Fluid (PN 653154) on the SIP and run the instrument for 5 minutes on FAST.
3. Place a sample tube containing 3 mls of 0.22 µm filtered, DI water on the SIP and run the instrument for 2 minutes on FAST.
4. From the Instrument menu in BD Accuri C6 Software, activate the manual decontamination fluid cycle by selecting “Run Decontamination Fluid Cycle”.
5. When the Decontamination Fluid Cycle is complete, turn off the BD Accuri C6
6. Remove tube of 0.22 µm filtered, DI water from SIP.
7. Disconnect the fluidic harness from the C6 and the fluid bottles, empty the four fluid bottles, and wipe down the outside of the instrument with 70% ethanol.
8. Pack the C6, fluid bottles and tray, fluidic harness, and power supply in the Pelican Case.

When disconnecting the fluidic harness from the cytometer, always disconnect from the bottle end of the harness first to prevent fluid leaks.

NOTE: Contact Accuri Technical Support if you are having difficulty performing this procedure due to instrument malfunction, or you need further guidance on the process.

5.3 Limited Warranty

Limited Warranty period is one (1) year from the earlier of, (i) first operation of the Products by Buyer or (ii) ten (10) days after shipment of the Products to Buyer.

This Limited Warranty covers software and firmware, and defects in material and workmanship under normal use and does not apply to ordinary wear and tear. For more detailed information, refer to the warranty information at www.AccuriCytometers.com.

5.4 Extended Warranties

Extended warranties are available. Visit www.AccuriCytometers.com for information.

5.5 Technical Support

For Technical Support, contact:

- **In the USA:**

  Accuri Cytometers Inc.
  173 Parkland Plaza
  Ann Arbor, MI 48103 USA
  Phone: +1.734.994.8000
  Fax: +1.734.994.8002

  Email: TechSupport@AccuriCytometers.com

- **Europe, Middle East and Africa**
  BD Biosciences
  Erembodegem-Dorp 86
  9320 Erembodegem
  Belgium
  Phone: +32 53 720 882

  Customer Support: bd_accuri@europe.bd.com
  Technical Support: techsupport@europe.bd.com

- **Canada**
  BD Biosciences
  2100 Derry Rd West, Suite 100
  Mississauga, Ontario
  Canada L5N 0B3

  Email: canada@bd.com

Direct Phone Numbers

Austria: 0810 101 807
Belgium: 078 166 050
Canada: 1.800.268.5430
Denmark: 8025 0622
Finland: 0800 915582
France: 0811 290 069
Germany: 0180 100 1732
Ireland: 1850 930 396
Italy: 840 999 926
Netherlands: 0900 0400 142
Norway: 800 17 382
Poland: 00800 121 4744

5.6 Ordering Information

Visit www.AccuriCytometers.com or www.BDBiosciences.com for a complete list of parts and reagents.

Regional Offices bdbiosciences.com/offices

Asia Pacific
Singapore
Tel 65.6861.0633
Fax 65.6860.1590
China
Tel 86.21.3210.4610
Fax 86.21.5292.5191
India
Tel 91.124.2383566
Fax 91.124.2383224/25/26

Australia/New Zealand
Australia
Toll Free: 1800 656 100
Tel 61.2.8875.7000
Fax 61.2.8875.7200
bd_anz@bd.com
New Zealand
Toll Free: 088 572.468
Tel 64.9.574.2468
Fax 64.9.571.2469
bd_anz@bd.com

Europe
BD Biosciences
Tel 32.2.400.98.95
Fax 32.2.401.70.94
bdbiosciences.com/eu
help.biosciences@europe.bd.com

Canada
BD Biosciences
Toll Free 888.259.0187
Tel 905.542.8028
Fax 888.229.9918
bdbiosciences.com/ca
canada@bd.com

Japan
Nippon Becton Dickinson
Toll Free 0120.8555.90
Tel 81.24.593.5405
Fax 81.24.593.5761

United States
BD Biosciences
Toll Free 877.232.8995
Fax 800.325.9637
bdbiosciences.com
answers@bd.com

Microsoft Office and Windows 7are trademarks of Microsoft Corporation. ©2012

For Research Use only. Not for diagnostic or therapeutic procedures. Class 1 Laser Product.

BD Accuri, BD Accuri C6 Software, BD Accuri CSampler Software, BD CSampler, and “Science is hard. Flow cytometry should be easy.” are trademarks of Becton Dickinson and Company. ©2012